SER. II, VOL. 11 *June, 1949* No. 8

TRANSACTIONS
OF
THE NEW YORK ACADEMY OF SCIENCES

Editor
ROY WALDO MINER

Associate Editor
PAUL LENIHAN

Contents

Experimental Studies of Adsorption on Aqueous Solutions. *By* ERIC HUTCHINSON 266

The Biological Significance of the Tissue Dose of Ionizing Radiations. *By* HARALD H. ROSSI 270

The Nature of Space and Visualization Abilities: Some Recent Findings Based on Factor-Analysis Studies. *By* WILLIAM B. MICHAEL 275

Influence of Nutrition on Growth and Morphology of the Dermatophytes. *By* LUCILLE GEORG 281

Copyright, 1949, by The New York Academy of Sciences

TRANSACTIONS of The New York Academy of Sciences, Series II, Volume 11, No. 8, June, 1949.
Entered as second-class matter December 12, 1947, at the post office at Baltimore, Maryland, under the act of August 24, 1912. This publication is distributed to members and is published monthly from November to June, inclusive, by The New York Academy of Sciences, Mt. Royal and Guilford Aves., Baltimore 2, Md. Editorial office: Seventy-Ninth Street and Central Park West, New York 24, N. Y.

Executive Director: EUNICE THOMAS MINER

UNIVERSITY OF MICHIGAN LIBRARIES

EXPERIMENTAL STUDIES OF ADSORPTION ON AQUEOUS SOLUTIONS

By ERIC HUTCHINSON†

Until recent years, the absorption theory of Gibbs was in good standing and acceptable to the majority of colloid chemists. This theory, which correlates the amounts of materials adsorbed at interfaces and surfaces with changes in the concentration or activity of the components of the phases whose common boundary forms the surface or interface, has been called into question a number of times of late.

In its most general form, as deduced by Gibbs,[1] the equation governing such adsorption may be written

$$-d\sigma = S^{\omega}\, dT + \sum_{1}^{k} \Gamma_i\, d\mu_i, \qquad (1)$$

where σ = surface tension,
S^{ω} = entropy per unit area of surface,
Γ_i = concentration of the i'th species per unit area, and
μ_i = chemical potential of the i'th species.

Γ_i, which is frequently, and misleadingly, referred to as a surface excess, may be envisaged in the following way. In FIGURE 1, the shaded area represents the region of discontinuity between the phases α and β. A plane, S, placed at an arbitrary level, serves to define the volumes V^{α} and V^{β} of two bulk phases. (Common sense indicates that this plane should be near, *i.e.*, within less than 100 Å, the physical boundary layer, which experiment shows to be definite to less than one or two molecular thicknesses at the most.[2]) If n_i moles of the i'th species are used in preparing the complete system, α, β, ω, and c_i^{α}, c_i^{β} are the concentrations of the i'th species in α and β, in the uniform portions of the bulk phases, then, assuming

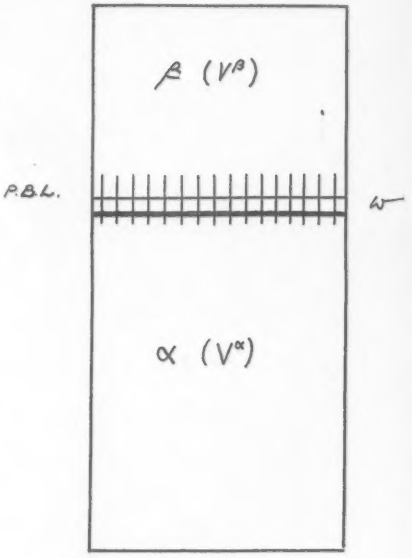

FIGURE 1.

that α and β are completely uniform up to the plane S,

$$n_i = n_i^{\alpha} + n_i^{\beta} + n_i^{\omega}$$
$$= c_i^{\alpha} V^{\alpha} + c_i^{\beta} V^{\beta} + n_i^{\omega};$$

$$i.e., \qquad n_i^{\omega} = n_i - c_i^{\alpha} V^{\alpha} - c_i^{\beta} V^{\beta}. \qquad (2)$$

This equation defines the *chemical content* of the surface layer.

Now, from EQUATION 1, it may be deduced that $\sigma = \sigma(K + 1$ intensive variables), but experience indicates that the surface or interfacial tension between two phases is

* The Section of Geology and Mineralogy held a meeting on May 2, 1949, at which Doctor H. D. Thompson, Department of Geology, Hunter College, N. Y., presented a paper entitled "Structure-Drainage in the Appalachian Mountains" an abstract of which will appear in the November issue of the TRANSACTIONS. This will be supplementary to his paper entitled "Drainage Evolution in the Appalachians of Pennsylvania" which won a George Frederick Kunz Prize at the Annual Meeting of the Academy in December, 1949, and which will appear shortly in the ANNALS of The New York Academy of Sciences, Volume 52, Article 2.

† Department of Chemistry, Fordham University, New York. This paper, illustrated with lantern slides, was presented at the meeting of the Section on May 3, 1949.

determined uniquely by the intensive state of the system. But the intensive state of a two phase system α and β, of K components in complete equilibrium, is determined by a number of intensive variables given by the phase rule,

$$f = K + 2 - \varphi,$$

where f = number of degrees of freedom

and φ = number of phases.

Hence, $f = K$ for a K component system. Therefore, it follows that

$\sigma = \sigma$ (K *independent intensive* variables)

and that, of the $K + 1$ intensive variables in the Gibbs adsorption equation, only K are independently variable.

In applying the equation to a particular problem, the experimental part consists in choosing some intensive variable x_j and measuring changes in σ caused by changes in x_j.
Then:-

$$-\frac{\delta\sigma}{\delta x_j} = S^{\omega}\frac{\delta T}{\delta x_j} + \sum_1^k \Gamma_i \frac{\delta \mu_i}{\delta x_j}. \qquad (3)$$

It can be shown[3] that a solution to EQUATION 3 is possible in practice only if temperature is chosen as one of the intensive variables; i.e., $\sigma = \sigma$ (T, $K - 1$ intensive variables, $x_2, \cdots x_k$). Then an array of equations is obtained—

$$\left.\begin{aligned} \sum_1^k \Gamma_i \frac{\delta \mu i}{\delta T} + S^{\omega} &= -\frac{\delta\sigma}{\delta T} \\ \sum_1^k \Gamma_i \frac{\delta \mu i}{\delta x_2} &= -\frac{\delta\sigma}{\delta x_2} \\ \sum_1^k \Gamma_i \frac{\delta \mu i}{\delta x_k} &= -\frac{\delta\sigma}{\delta x_k} \end{aligned}\right\}, \qquad (4)$$

in which there are $K + 1$ unknowns, viz. S^{ω}, Γ_1, $\Gamma_2, \cdots \Gamma_k$, for the solution of which there are only K simultaneous equations. Thus, a complete solution is possible only when a further, auxiliary equation is employed. It may be shown[3] that such an auxiliary equation can be made to define uniquely the position of the plane S, the "Gibbs surface," referred to earlier. Any equation of the type

$$\Phi(j_1, \cdots j_n, b_1 \cdots b_\theta) = 0$$

may be used to define the position of the Gibbs surface, where j_1 and $\cdots j_n$ are any set of functions of the state of $\alpha\beta\omega$, at least one of which must be variable with the position of the Gibbs' surface, and b_1 and $\cdots b_\theta$ are any set of functions of the state of $\alpha\beta\omega$ which are not variable with the position of the Gibbs' surface and may be constants.

Any equation Φ may be used which is continuous and dimensionally correct. Thus, for example, Guggenheim and Adam[4] suggest

$$\Gamma_1 V_1^{\alpha} + \Gamma_2 V_2^{\alpha} = 0 \qquad (K = 2)$$

$$\Gamma_1 N_1^{\alpha} + \Gamma_2 N_2^{\alpha} = 0 \qquad (K = 2)$$

where V^{α} and N^{α} are, respectively, partial molal volumes and mole fractions. Gibbs himself used the equation $\Gamma_{\text{solvent}} = 0$, which, although easy to apply, is difficult to envisage in physical terms and has been accorded an altogether undeserved importance by physical chemists. The equation which is used may be designed to incorporate any physical interpretation of the nature of the adsorbed film, as, for example, the hypothesis that the adsorbed film is monomolecular.[4]

By historical accident, most experimental investigations of the adsorption equation have been restricted to two-component aqueous solutions. For such solutions, the equation takes a very simple form if the Gibbs convention $\Gamma_{\text{water}} = 0$ is used. Under these conditions, choosing as the intensive variables temperature and concentration, activity, or mole fraction, the following solutions are obtained:

$$-\frac{\delta\sigma}{\delta c_s} = \Gamma_s \frac{\delta \mu s}{\delta c_s}, \qquad (5.1)$$

$$-\frac{\delta\sigma}{\delta a_s} = \Gamma_s \frac{\delta \mu s}{\delta a_s}, \qquad (5.2)$$

UNIVERSITY OF MICHIGAN LIBRARIES

and

$$-\frac{\delta\sigma}{\delta N_s} = \Gamma_s \frac{\delta\mu_S}{\delta N_s}. \qquad (5.3)$$

Provided $\frac{\delta\mu_S}{\delta c_s}$, $\frac{\delta\mu_S}{\delta a_s}$, and $\frac{\delta\mu_S}{\delta N_s}$ can be evaluated, as can usually be done, then Γ_s may be calculated.

Experiments designed to verify the Gibbs equation were reported by Donnan,[5] McBain,[6] and others,[7] and many yielded data for *directly determined* Γ's in considerable quantitative disagreement with the Gibbs' equation but in qualitative agreement in that compounds, which in aqueous solution decreased the surface tension of water $\left(\frac{\delta\sigma}{\delta c_s} < 0\right)$, were found to yield positive Γ's.

McBain and Swain,[8] in a refined experimental approach to the direct determination of Γ_s by the "microtome" method, found excellent agreement with the Gibbs equation for a number of simple solutes such as phenol and cinnamic acid. Thus, there appears to be very satisfactory agreement between the observed data and those calculated from the Gibbs equation for a number of compounds on which careful experiments have been performed.

From about 1937 onwards, however, a number of anomalous effects were observed which appeared to be in direct conflict with the Gibbs theory. A very large class of surface active materials, which are generally classified as detergents, were reported to yield, in aqueous solution, surface tension concentration curves exhibiting a minimum. These minima were found to occur at values of the surface tension between $25 \sim 40$ dynes/cm. Experience clearly indicates that, with such large depressions of the surface tension of water, adsorbed material is present on the surface, and yet inspection of the Gibbs equation would appear to indicate that at the minimum, where $\frac{\delta\sigma}{\delta c_s} = 0$, Γ_s should also be zero. Hence, there seemed to be a serious discrepancy between thermodynamic and experimental fact. A number of authors[9] suggested that the Gibbs equation is incorrect and needs to be modified. This is a somewhat startling suggestion, in view of the fact that the Gibbs equation is based on classical thermodynamic concepts, not called into question in other contexts.

One highly significant factor observed with materials exhibiting minima in surface tension curves was that all such compounds become colloidal in solution above a certain critical concentration, forming micelles of the type studied by McBain and his school.[10] Moreover, the minima were observed to occur at or near the critical concentration for micelle formation.

A significant advance was made as a result of an observation by Miles and Shedlovsky[11] to the effect that, when a sample of sodium dodecyl sulfate (normally observed to give a minimum surface tension curve) is purified by exhaustive extraction, no minimum is observed. Moreover, the minimum is restored by the addition of small quantities of impurities such as dodecyl alcohol or homologs of the detergents. Extremely small amounts of alcohol, 0.05 per cent, are sufficient to cause the minimum to reappear. These observations have been extended to a number of alkyl sulfates and sulfonates by Ross and Shedlovsky.[12]

It appears highly probably that all minima may be explained in terms of small quantities of impurities. Further, the poor reproducibility of the results of independent investigators becomes understandable as a result of the extreme susceptibility of these systems to small traces of impurity.

The qualitative observations of Miles and Shedlovsky have been confirmed and demonstrated quantitatively by the author.[13, 14] It is clear that, when it contains only small traces of highly surface active impurity, a solute of the kind represented by sodium dodecyl sulfate cannot be treated, even approximately, as a single thermodynamic component. A recent theoretical investigation of the Gibbs adsorp-

tion equation as applied to multi-component systems[3] makes possible the calculation of the Γ's for both the main solute component, say sodium dodecyl sulfate, and an impurity, such as an alcohol.

Surface tension curves of a series of pure alkyl sulfates in aqueous solution, in the presence and absence of straight chain alcohols and homologous alkyl sulfates, have been determined in the author's laboratory,[14] from which Γ's for the various components have been determined.

It is found that, at concentrations of about 1 per cent of that of the detergent, a straight chain alcohol comprises about 50∼80 per cent of the mixed adsorbed film in the presence of an alkyl sulfate containing the same number of carbon atoms, demonstrating the very much greater surface activity of the alcohol. The addition of the alcohol to an alkyl sulfate solution does not cause a too great change in the absolute value of Γ for the sulfate; the alcohol rather *enters* the adsorbed film than *replaces* alkyl sulfate molecules. Thus, the presence of a small amount of alcohol causes an *additional* depression of the surface tension, so that, below the 'critical' concentration, the surface tension curve of a contaminated alkyl sulfate solution is much lower than that of a solution of the pure material. Such phenomena, therefore, explain the large depressions of surface tension observed below the critical concentration with impure materials; but the problem of the minimum still remains.

Now, it is highly significant that all materials which exhibit minima form colloidal micelles above the critical concentration. The work of McBain and his school[10] shows that such micelles can "solubilize" materials, often in considerable amounts up to mole/mole ratios of 0.2 for the solubilized material and solubilizer. This process is thermodynamically reversible and may be considered in some respects as an ordinary distribution phenomenon, the solubilized molecule being distributed between free aqueous solution and the hydrocarbon-like interior of the micelle. With materials such as long-chain alcohols the equilibrium distribution is shifted strongly in favor of the micelle phase, so that the amount of alcohol in free solution is much less than that corresponding to the stoichiometric proportion of the alcohol in the whole system. Hence, the activity of the alcohol in aqueous solution undergoes a rapid decrease as the concentration of the detergent is increased above the critical concentration and micelles form. Thus, the contribution of the alcohol to the adsorbed film is drastically and rapidly reduced in the region just above the critical concentration, with a consequent increase in the surface tension of the solution to values corresponding closely to those of solutions of the pure detergent. Similar arguments may be applied to any other similar surface active compounds containing impurities. Clearly, the rate of increase of surface tension beyond the minimum should depend on the surface activity of the impurity and on the relative ease of solubilization of the impurity. Experiments are being planned to verify these points.

Hence, it appears that, if proper care is exercised in the purification of materials, no discrepancies with the Gibbs adsorption theory occur for two-component systems. Further, in multi-component systems, if the number of components is known, a complete solution of the complex surface tension phenomena is possible without benefit of additional postulates beyond those originally proposed by Gibbs.

REFERENCES

1. GIBBS, J. W. 1948. Collected Works. Yale University Press. New Haven.
2. RAYLEIGH, J. W. 1892. Phil. Mag. **33:** 1.
3. KOENIG, F. & E. HUTCHINSON. To be published.
4. GUGGENHEIM, E. A. & N. K. ADAM. 1933. Proc. Roy. Soc. **139** (A): 218.
5. DONNAN, F. G. & J. T. BARKER. 1911. Ibid. **85** (A): 557.
6. MCBAIN, J. W. & G. P. DAVIES. 1927. J. Am. Chem. Soc. **49:** 2230.
7. GIBBY, C. W. & C. C. ADDISON. 1936. J. Chem. Soc. **119.**

8. McBain, J. W. & R. C. Swain. 1936. Proc. Roy. Soc. **154** (A): 608.
9. McBain, J. W. & G. T. Mills. 1939. Reports of Progress in Physics. **V**: 30.
10. McBain, J. W. 1942. Advances in Colloid Science. Interscience. New York.
11. Miles, G. D. & L. Shedlovsky. 1944. J. Phys. Chem. **48**: 57.
12. Shedlovsky, L. & J. Ross. 1949. J. Coll. Sci. **4**: 25.
13. Hutchinson, E. 1948. Ibid. **3**: 413.
14. Hutchinson, E. & R. J. Conan. To be published.

SECTION OF BIOLOGY

THE BIOLOGICAL SIGNIFICANCE OF THE TISSUE DOSE OF IONIZING RADIATIONS

By HARALD H. ROSSI*

Although the tissue dose of ionizing radiations is, today, one of the most fundamental quantities in radiobiology, its units, as well as measurements in terms of such units, are in a rather unsettled state, except in the case of X-rays and gamma rays below a few million volts energy where determinations in terms of the roentgen are fairly well standardized. While the delay in the case of the other radiations is certainly due to the relative novelty of the problem, the matter is aggravated because of its physical complexity. Certain aspects of the problem will be presented here, with special regard to the problem of a quantitative measurement of radiation and the question as to how far the result of such a measurement serves to define the conditions under which a radiobiological experiment is performed.

Any such experiment requires some sort of dosimetry. The most primitive type, perhaps, is the one in which there is merely a question as to whether any radiation at all is reaching the object which is supposed to be irradiated. This is by no means as simple a matter as might appear at first glance. It is true that no experimenter having any knowledge of the field would expect to investigate radiobiological effects in regions where, for obvious physical reasons, there is no radiation. Thus, the beta rays of radioactive phosphorus, which penetrate only a few millimeters of tissue, would certainly not be employed as external sources for whole body irradiation of a mouse. On the other hand, they might be employed internally, and then the question as to whether any organ of the animal receives any radiation will depend on the way the isotope is administered—whether it is fed or injected, whether it is in a soluble or insoluble compound, *etc.* In other words, the physical dose will depend greatly on biochemical and physiological factors; and, under these circumstances, dosimetry, even of the vaguest kind, is by no means simple.

Dosimetry must be fairly exact, of course, when the conditions of a biological experiment are to be described so that work performed by one biologist may be repeated by another. In this case, the amount of radiation involved should be given with at least the accuracy with which the biological effect may be ascertained. In determinations of L.D. 50 (the dose required to kill half the animals of a given group), a five or ten per cent error in dose determination will usually be small compared with individual or statistical variations. However, in cases where good statistics and virtually no individual variations exist, as for instance in certain genetic work, dosimetry within a few per cent is quite desirable.

Fairly accurate dosimetry is also required when the effects of different radiations are to be compared. This brings us to the question of a unit of tissue dose, which may be used for any ionizing radiation, as contrasted with the roentgen, which is restricted to X- and gamma rays. A

* Radiological Research Laboratory, Columbia University, New York. This paper, illustrated with lantern slides, was presented at the meeting of the Section on May 9, 1949.

discussion of this question must be preceded by a brief account of the physical processes which take place when ionizing radiation interacts with matter.

Ionization of matter is ultimately always carried out by electrically charged particles of high velocity, even though the primary radiation may consist of uncharged particles or quanta. The most important practical case in which charged particles are used directly in the irradiation of biological material is, today, probably in the use of beta ray-emitting isotopes which are used as "distributed sources," i.e., are, through injection or other means of incorporation, distributed throughout the biological material irradiated. The high-speed electrons emitted in the decay of the radioactive material penetrate from a few microns to several millimeters of tissue, depending on the kind of isotope used. If the objects to be irradiated are sufficiently small (such as unicellular organisms), the isotope may be used in external irradiation. Electrons, and even positively charged ions (protons, alpha rays *etc.*), may in some modern accelerators be made to acquire such energies that they can penetrate several inches of tissue, but these installations are thus far rarely available to the biologist. If relatively uniform external irradiation of large masses of tissue is required, use is made of primary radiations which are not ionizing *per se* and are much less strongly absorbed than ionizing particles. The interaction of these radiations with matter gives rise to secondary charged particles, which will be the more uniformly distributed in the affected tissues the less the primary "neutral" radiation is absorbed in passing through these tissues.

FIGURE 1 gives a summary of the possible reactions taking place when these radiations interact with matter. While, for the sake of completeness, all possible types of reactions are listed, only some of them are of importance when tissue is exposed to the radiation beams which are usually available.

Without going into the details of any of these processes, it might merely be pointed out that X- and gamma rays will, in all practical cases, give rise to electrons, while the only directly ionizing particles liberated by neutrons are positively charged nuclei or nuclear fragments. These differ from electrons by losing their energy much more rapidly when passing through tissue, but the mechanism of energy loss is virtually the same.

The electromagnetic fields set up by the motion of these particles cause the ejection of electrons from atoms in the matter traversed, and this leads to a trail of positive and negative ions (the latter formed by attachment of the electrons to neutral atoms) in the wake of the ionizing particle.

It is well to point out, however, that not all the energy of an ionizing particle will be spent in ionization. When an ionizing particle traverses matter, only about one half of its energy is ever spent in the production of ions. The other half is immediately expended in excitation of molecules; i.e., in displacement rather than actual ejection of electrons. Since the energy required for an excitation is generally considerably lower than the energy spent in an ionization, excited atoms will far outnumber ionized atoms. Furthermore, the excited atoms as well as the ionized atoms will radiate as they return to their normal state, and the emitted radiations will, in turn, cause further excitation. The energy of the radiations decreases steadily, and, finally, most of the energy is degraded into heat. After a short time, this heat diffuses throughout the affected material and leads in all practical cases to a negligible temperature rise. Before this ultimate dissipation, however, the heat is concentrated in very small volumes, and the temperatures (if one can speak of such on such a microscopic scale) are very considerable. These considerations form the substance of the "point heat" theory of Dessauer, a concept which is revived whenever the biological action of radiation is discussed.

While it is customary to attribute the biological effect of ionizing radiations to actual ionizations, there can be little doubt

that excitations play at least some part in the process. This is suggested by experimentation with ultraviolet light, which can only initiate excitations and which often shows effects similar to the ones caused by ionizing radiations. It is believed that more excitations than ionizations are required to obtain most biological effects. However, beyond this rather vague realization, little is known. The absence of experimental information is paralleled by our inability to predict from theoretical grounds what processes should take place. In fact, even the question as to just what happens when radiation passes through pure water has not been entirely solved experimentally, and it would be quite unreasonable to expect that it will ever be

INTERACTIONS BETWEEN NEUTRAL RADIATIONS AND MATTER

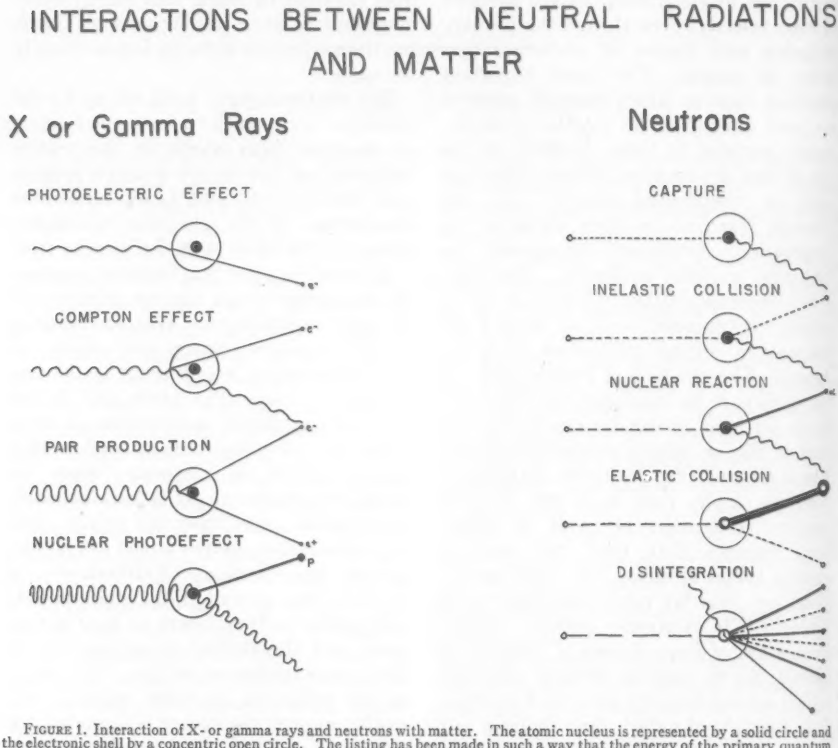

FIGURE 1. Interaction of X- or gamma rays and neutrons with matter. The atomic nucleus is represented by a solid circle and the electronic shell by a concentric open circle. The listing has been made in such a way that the energy of the primary quantum or neutron must *usually* be higher to initiate a reaction which is further down a column. The individual reactions are:

X- or Gamma Rays:

Photoelectric Effect: Absorption of a quantum and emission of an electron.

Compton Effect: The quantum is scattered with longer wave length (lower energy) by an electron which receives energy. (Scattering without change in wave length, another possible process, has been excluded, since it does not contribute to a dose.)

Pair production: Creation of an electron-positron pair near the nucleus.

Nuclear photo effect: A nuclear transformation initiated by a quantum.

The last two reactions are, at present, of no practical significance in biological experimentation.

Neutrons:

Capture: Absorption of a neutron usually followed by emission of a gamma ray.

Inelastic collision: Absorption of a neutron and re-emission at lower energy. Usually accompanied by emission of a gamma ray.

Nuclear Reaction: Usually accompanied by emission of a light nuclear fragment and a gamma ray.

Elastic Collision: A fraction of the primary neutron energy is imparted to a recoiling nucleus.

Disintegration: Splitting up of a nucleus into small fragments.

The last of these processes occurs only at very high neutron energies (above about 25 MEV).

settled by a theoretical physicist. The variety and complexity of possible reactions taking place in so non-homogeneous a material as protoplasm must be enormous, and the possible number of excitations, bond breakages, bond shifts, recombinations, and back reactions must certainly be considered as running into the billions. Obviously, no physical unit of measurement can be devised which takes into account any detailed aspect of these processes.

There are only two quantities which may conceivably be used as a basis for a physical unit. One is the number of ion pairs created in the ionization process; the other is the total energy imparted per unit mass of matter irradiated. The first of these possibilities must be ruled out because, at present, we know of no way to measure ionization in a solid. We can measure ionization only in gases. However, we can deduce energy loss in a solid by measuring ionization in an enclosed gas, provided certain physical and geometrical conditions are met. The ionization in the solid cannot be accurately inferred from such measurements, although there is reason to assume that it is fairly proportionate to energy absorbed for any ionizing particle and that the absorption of one erg of energy is accompanied by about 2×10^{10} ionizations.

Energy absorbed per gram of tissue may thus be considered a satisfactory basis for a unit by both the biologist and the physicist. It seems likely that any international unit for ionizing radiation will either be based directly on ergs absorbed per gram of irradiated substance or be closely related to that quantity. At present, the most widely used (unofficial) unit of ionizing radiation is the rep (H. M. Parker), which is usually understood to be that amount of ionizing radiation which liberates 83 ergs per gram of tissue. This is roughly equal to the energy imparted to a gram of tissue by one roentgen of X-rays; a quantity which varies from about 85 to 96 ergs, depending on the wave length of the radiation. The rep is used to quantify the radiations to which the roentgen does not apply (charged particles and neutrons).

A good deal of progress has been made in the last few years in the actual measurement of tissue doses in terms of energy units. A feature of some of this work has been a tendency to make the measuring device approach the characteristics of actual tissue to a considerable extent. In fact, we have, in our laboratory, under the direction of Dr. Failla, developed a material having the same *atomic* composition as average soft tissue. This material is used in the construction of ionization chambers which have the same "response" as tissue, and the ionization observed in such chambers is a good measure of energy absorbed in tissue.

The questions arise as to just what value the results of such measurements have for the biologist and if the physicist can be of further assistance after he has supplied them.

The first point to keep in mind is that, in practice, both the roentgen and the rep are time and space integrals. That is, they give the dose received without supplying information as to when and where it was received.

The time dependence will be of importance only for those effects for which there is recovery. We know that, in many cases, a "fractionated dose" will be less effective than an equal single dose, because there may be a certain amount of recovery during the intervals between the repeated irradiations. Recovery may also occur during a protracted exposure at low irradiation levels, provided the duration of the exposure is comparable with the time required for significant recovery. On the other hand, the dose rate will be unimportant if the duration of exposure is short compared with rate of recovery. Since most sources of radiation have a rather constant output, measurement of the dose received during a certain time may be expected to yield a dose rate which

is substantially constant for any time interval.

Fluctuations of the spatial distribution of energy absorption may be of several types. The most basic of these is due to the fact that the energy absorption is ultimately a statistical process. While it is customary to speak of a dose at a "point," an actual measurement can, of course, be made only with equipment of finite size, and the result of the measurement represents the average reaction of a finite amount of matter. But, even if it were possible to measure the dose received in an infinitesimal volume such as an individual atom, the outcome of the measurement would be quite trivial.

Even a dose of 100,000 r will ionize about one atom in a million, and the "dose" received by any given atom will usually be zero; and, in the rare case when it is not, it will be enormous. There is evidently no way of overcoming this difficulty in the case where it interferes with biological experimentation. Thus, in a study of mutation rate, where the dose must be limited to values which do not kill the genetic material, the probability of scoring a "hit" on any particular kind of gene will usually turn out to be rather small, and only work done with a great amount of material will have statistical significance.

Since the tissue dose is initially imparted along the tracks of ionizing particles, a given volume of tissue will be ionized twice, if at all, when its diameter is larger than the ion spacing along the track. If the biological effect under investigation requires more than one ionization in the volume, a heavily ionizing radiation will obviously be far more effective in bringing about the effect. On the other hand, if only one ionization is required, excessive ionization will lead to a "waste" of available ions, and lightly ionizing particles, such as electrons, will be more effective. Reasoning along these lines has lead to an important hypothesis in radiobiology: the "target-theory" of biological action. While it is not the purpose of this paper to analyze the details or merits of that theory, it is plain that ion density is an important characteristic of ionizing radiations. This is obvious from the well-known fact that the biological effects of equal energy doses vary considerably from lightly to heavily ionizing radiations. We have seen that X-rays give rise to sparsely ionizing electrons, while neutrons produce predominantly heavily ionizing secondaries. The fact that fast neutrons are about seven times more lethal to a mammal than X-rays shows how important the difference in ion density is. We know that the ion-spacing, as well as the spacing of excited atoms (which are relatively distributed in the same ratio), is about a hundred times larger in the tracks of electrons liberated by hard X-rays than along the paths of neutron recoils; but this figure is very rough indeed. The ion density increases along the track of any ionizing particle, and, in fact, the ion density near the end of electron tracks often may be higher than in the initial portions of a proton track. Obviously, ion density is an important factor which cannot be easily expressed in a quantitative way, and, while it is possible for instance, to say that a certain energy dose was delivered by "fast neutrons," the actual information on spatial distributions of ionization is quite vague.

Varying spatial distribution of ionization on a larger scale will result from a non-uniform deposition of radioactive isotopes in tissues. Effects of this type may be strikingly demonstrated by the use of autoradiographs in which the radiation emitted by radioactive substances, which are deposited in a tissue section, are made to affect a photographic emulsion. Here too, wide variation in distribution may be observed, and the tissue dose for a *uniformly* distributed isotope, the quantity commonly supplied by the physicist, is often of no value whatsoever.

It should be pointed out, however, that a special analysis of a given problem can be of help in many cases where a simple value

of average dose is useless. A large body of statistics has been created, for instance, which may be used to evaluate biological effects of single ionizations despite the randomness of such events. Fairly good values of ion spacings, average or extreme, may be obtained by studies of radiation fields, and the problem of non-uniformly deposited isotopes may be attacked either by experimental determinations with simulated geometries or, sometimes, even by outright computation. Thus, the question as to whether radioactive phosphorus is a good agent in the treatment of leukemia because it concentrates four or five times more in the white cell than in the serum may be answered in the negative on the basis of fairly simple calculations.

Therefore, in summary, it may be emphasized that, while the biologist may, in certain cases, derive little value from conventional dosimetry, he will usually obtain significant data by collaborating on special problems with the physicist. The results of such collaboration certainly will contribute to the progress of science.

SECTION OF PSYCHOLOGY

THE NATURE OF SPACE AND VISUALIZATION ABILITIES: SOME RECENT FINDINGS BASED ON FACTOR-ANALYSIS STUDIES*

By WILLIAM B. MICHAEL†

History of Problem

Contrary to the traditional practices by which many of the older psychological concepts regarding the nature of mental abilities have been formulated, the construct of a spatial and/or visualization ability has been developed inductively through the medium of factor analysis. In a well-known investigation in which he reported the isolation and a psychologically meaningful identification of seven primary mental abilities, Thurstone[12] described a factor which resembled a group factor reported by Kelley[8] in earlier studies. This same factor appeared in later analyses reported by Thurstone[13] and by Thurstone and Thurstone.[14]

During World War II, several factorial studies carried out by members of the psychological research units of the Army Air Forces revealed that the variance associated with Thurstone's primary spatial-visualization ability could be separated into two apparently independent factors.[2] These two factors were identified as spatial-relations and visualization abilities respectively, abbreviated as S_1 and V_2. In fact, two other space factors (S_2 and S_3), which were not too readily interpretable, and a factor tentatively indentified as visual memory, appeared in several analyses. The fact that the spatial-relations ability represented one of the two most valid factors in the selection of pilots (as evidenced by its high weight in the pilot pass-fail criterion) is indicative of its practical importance.[2, 9] This factor appeared to represent primarily an ability to make accurate discriminations of the spatial relations of objects with reference to the location of the human body. The factor identified as visualization involved the process of imagining changes in positions of visual objects and required the subject mentally to twist, turn, or rotate these objects from one position to another. (An elaboration of the psychological meaning of these two factors is to follow).

* The writer wishes to express his gratitude to the Social Science Research Council for kindly making available a grant-in-aid, without which completion of three studies to be described would not have been possible. Grateful acknowledgment is made to Drs. J. P. Guilford and Wayne S. Zimmerman for many valuable suggestions during the course of the investigations.

† Department of Psychology, Princeton University, Princeton, N. J. This paper was presented before the meeting of the Section on May 16, 1949.

The Problem

The primary purpose of this paper embraces two objectives: (1) to summarize the results of three postwar factor-analysis studies (undertaken by the writer) that were planned to test the validity of two hypotheses concerning differences in the psychological properties of the factors of spatial relations and visualization, and (2) to review the findings regarding spatial and visualization abilities in two factorial studies by two investigators[1, 16] who have reanalyzed data presented by Thurstone in his pioneer study.[12] A secondary purpose, which is an outgrowth of the first objective, is to indicate whether tests of the type employed by the AAF and Thurstone's tests have, in common, factors that can be identified to be the same. In other words, a summary is to be presented of the results of three studies which serve as a check upon the belief that the AAF factors and Thurstone's primary mental abilities are essentially identical. Special attention, of course, is to be directed toward Thurstone's spatial factor.

Investigations Planned to Test Two Hypotheses Regarding The Nature of Spatial-Relations and Visualization Factors

General plan followed. Within each of the test batteries administered, two groups of tests were incorporated which were believed to be representative of the psychological processes described in the hypothetical statements to follow concerning the nature of the spatial-relations and visualization abilities. Introspection was freely employed as a means of finding a group of tests which appeared to represent a common task. To these two groups of tests were added several reference tests of fairly well-known factorial content to assist in the identification of those portions of variance in each test of a given group associated with other intellectual factors. These reference tests, it was thought, would tend to indicate the degree of purity of each test within a group intended to measure an hypothesized function. Moreover, the incorporation of tests not planned to yield evidence regarding the tenability of the hypotheses would serve to reduce the amount of specific variance in the spatial-visualization tests considerably.

A sufficient, though not necessary, condition for the tenability of each of the hypotheses would be that a different factor is defined in the factor-analysis procedure by each of the two groups of tests. Should one or more tests within a given group contain a substantial amount of variance in the factor identified by the second group of tests or in one of the reference tests, such a finding would tend to make the evidence for the hypothesis corresponding to the first group tests less convincing but not necessarily lacking.

The samples. The two hypotheses to be tested were the same for all three samples studied. One group consisted of 360 male subjects of Rutgers University who were taking an introductory course in psychology. Of the group, 220 were freshmen and sophomores, and 140 were juniors and seniors majoring in nearly every department of the University. Slightly more than half were veterans. The age of the subjects varied from 16 to 34. The median age was 22 years. Two high school samples, one consisting of 151 boys and the other of 139 girls, in the twelfth grade of John Muir Junior College in Pasadena, California, were also employed. The age of these subjects ranged for each sex group for 15 to 20, the median age being 17 years in each sample.

The tests. To the college group, fourteen tests were administered: six factor tests of the Guilford-Zimmerman *Aptitude Survey* (*Verbal Comprehension*, *General Reasoning*, *Numerical Operations*, *Perceptual Speed*, *Spatial Orientation*, and *Spatial Visualization*) and eight tests once employed by Thurstone* in his experimental studies (*Completion*, *Number Series*, *Identical Forms*, *Cubes*, *Flags*, *Punched Holes*, *Pattern Ana-*

* The writer wishes to express his appreciation to Professor Thurstone for his permission to reproduce several of his tests for inclusion in the batteries employed in these three studies.

logies, and *Form Board*). The six tests of the Guilford-Zimmerman *Aptitude Survey* were of a type similar to those used in AAF, although they represented a completely new set of tests with many improvements.[6,7] To the two high school samples, the same six tests of the Guilford-Zimmerman *Aptitude Survey* were given. Four of Thurstone's experimental tests, *Cubes*, *Form Board*, *Punched Holes*, and *Identical Forms* were used along with four tests from the Thurstone's battery of *Primary Mental Abilities* (long forms): *Number*, *Verbal*, *Space*, and *Reasoning*. A part of a test by Wrightstone titled *Spatial Relations* was also used, although the content of the test items suggests that it probably has a larger proportion of its variance in visualization than in spatial-relations.

For the college sample, the two groups of tests used to check upon the validity of the two hypotheses regarding the nature of spatial-relations and visualization factors consisted respectively of (1) *Cubes*, *Flags*, and *Spatial Orientation*, and (2) *Form Board*, *Punched Holes*, and *Spatial Visualization*. For the two high school samples, the group of tests intended to furnish evidence regarding the tenability of the first hypothesis as to the nature of spatial-relations factor consisted of *Cubes*, *Spatial Orientation*, and *Space* (by the Thurstones), and the group of tests chosen in conjunction with the factor of visualization included *Form Board*, *Punched Holes*, *Spatial Visualization*, and perhaps *Spatial Relations* (by Wrightstone).

Hypotheses. In a recent article[10] in which the findings for the college sample were reported, the two hypotheses regarding the nature of the spatial-relations and visualization factors were formulated in considerable detail. Accompanying the statement of each hypothesis was a description of the manner in which each test in the group of tests selected was thought to conform to the type of task relevant to the corresponding hypothesis. Since a clear understanding of the hypotheses is essential for subsequent interpretation of the results, the recent formulations are quoted in their entirety:

> The factor of spatial relations was hypothesized to represent the ability to comprehend the *arrangement* of elements within a visual stimulus pattern primarily with reference to the human body. Thus, an important implication in the ability to perceive spatial arrangements is that the subject is able to distinguish whether one object is higher or lower, left or right, or nearer or farther than another within the same field. Through the presentation of two simulated views of a stimulus pattern, a test item may be constructed such that there is a systematic relationship between the order of elements within the first spatial pattern (the stimulus component of a test item) and the order of elements within the second pattern (the response component of a test item).
>
> For example, in Thurstone's *Cubes* test the examinee is asked to recognize whether the designs on the sides of a second cube can hold the same relationship to one another as they do on the first cube. By noticing within each cube the left-right, top-bottom, and front-back interrelationships of the faces, the subject is able in each item to refer the locations of three designs on three exposed faces of one cube to the locations of designs on the faces of the other cube. In Thurstone's *Flags* test the examinee is required to tell whether the exposed faces of the American flags of identical size can represent the same side of the flag. Relating corresponding left-right and top-bottom boundaries (outlines) of the two flags appears to be an important aspect of the solution. Similarly, in Guilford and Zimmerman's test of *Spatial Orientation* a premium is placed upon the examinee's maintaining the correct relationship of objects to one another in background scenery that has been viewed twice from a motorboat—first before and then after its prow has moved up or down and/or left or right. In the test the examinee is asked to determine the relative amount and direction of movement of the boat corresponding to changes in the two views of the background setting.
>
> The factor of visualization was hypothesized to represent an ability that requires the mental *manipulation* of visual images. In contrast to another factor identified as visual memory (*2*), which appears to be a static or reproductive form of visualization, the factor referred to as visual manipulation, or simply visualization, is dynamic. This visual manipulative ability appears to be present in the solution of problems in which the individual finds it necessary mentally to move, rotate, turn, twist, or invert one or more objects. Following the performance of the presented manipulation the individual is required to recognize the new position, location, or changed appearance of the object or objects.
>
> Three tests selected to yield evidence for the second hypothesis included two by Thurstone, *Punched Holes* and *Form Board*, and one by Guilford and Zimmerman, *Spatial Visualization*. In the test of *Punched Holes* the examinee is presented a symbolic representation of a folded sheet of paper into which one or more holes have been punched and is required to imagine where the holes will be when the sheet is unfolded. In the second Thurstone test the

examinee apparently finds it necessary in each item mentally to turn, rotate, or invert two or more flat geometric figures in such a way that they can be placed together to fit within the outline of a larger geometric figure. In each of the tests, the examinee is asked to record the final positions respectively of the holes and of the geometric figures. In the test of *Spatial Visualization* the subject is required mentally to turn, tilt, or rotate a three dimensional object—an alarm clock—drawn on a sheet of paper into a final position according to written instructions. As alternative responses the pictures of the clock are presented in five positions, one of which is correct.

Whereas in the two Thurstone tests the examinee is required to draw in his solution to the problem, in the third test he merely selects as his solution one of five choices presented. It is quite likely that in addition to measuring visual manipulative ability other factors are involved in the three tests—factors reflecting the manner in which responses to the items are recorded.

Another important difference in the nature of the psychological processes hypothesized for the spatial-relations and visualization factors was that of speed of response. As indicated by findings in the AAF Aviation Psychology Program, the tests thought to measure the spatial-relations factor were administered with fairly short time limits, but those tests thought to measure visualization were given with fairly liberal time allowances. The spatial relations factor was considered to demand a fairly rapid decision on the part of the examinee as to the spatial position of objects with reference to his own location; whereas, the visualization factor was believed to be represented in problems requiring a more deliberate and less automatic approach. In part such a distinction may be a function of the complexity of a task (i.e., the number of steps entering into the performance of an item), the more complex tasks requiring visualization for their solution.[10]

(The only additional point which should be made in the instance of the two high school samples concerns the test *Space* by the Thurstones. This test, consisting of three exercises, or sub-tests: *Flags*, *Figures*, and *Cards*, should be expected to be loaded primarily on the spatial-relations factor, inasmuch as the items appear to represent essentially the same type of task found in Thurstone's experimental test *Flags*.)

Factor analyses. The Thurstone centroid method of factoring was employed for the three matrices of test intercorrelations. In order that a psychologically meaningful interpretation of the factors might be realized, the axes were rotated by pairs to positions satisfying Thurstone's criteria of simple structure and positive manifold. A graphical method devised by Zimmerman[15] was employed in rotation.[10] In the three analyses, six rotated factors were meaningfully identified as verbal comprehension (V), general reasoning (R), numerical facility (N), perceptual speed (P), spatial-relations (S), and visualization (V_z). In each analysis, two other factors appeared that could not be readily interpreted, although work upon the two analyses for two high school samples is still in progress, with the view that certain minor adjustments may bring about a plausible interpretation for at least one more factor. With the exception of the spatial-relations and visualization factors, the other four factors V, R, N, and P appeared to be identical with those previously found by Thurstone.

Evidence regarding the validity of the two hypotheses. The hypotheses regarding the nature of spatial-relations and visualization abilities were upheld, since, in the main, variances associated with these two factors were limited to the respective groups of tests initially inserted within the test batteries to bring out the factors. However, it should be pointed out that in the three analyses some of the tests in these groups tended to be complex factorially—that is, they were weighted in both the spatial-relations and visualization factors. Nevertheless, the larger component of variance in a complex test was in the factor common to the group in which that test appeared.

Introspective reports obtained from several of the subjects revealed that, for a given test, some individuals depended upon one psychological process— others, upon another. For example, in the *Cubes* test, many a subject reported that he matched up the locations of the sides of the cubes with respect to his own position—left face in the second cube with what was front face or back face or right face in second cube, top face with what was front face or right face or left face. Such a process would be considered to represent a spatial-relations ability. On the other hand,

if an individual reported that he picked up the first cube as if it were a block or die and proceeded to rotate it into a final position which matched (or could not match) the second cube, then the process of visualization would be dominant. In their introspections, some individuals revealed that they put to use both processes.

Inasmuch as the results based on data obtained from the high school samples studied are still somewhat tentative at the time of the writing of this paper, detailed information regarding the magnitude of the factor loadings will not be presented. Suffice it to say, the two factors of spatial-relations and visualization were readily identified. The two hypotheses were upheld. The pattern of loadings in the two groups of tests resembles closely that found in the two similiar groups of tests employed in the college sample.

The factor loadings to be reported will be confined to those in the six key tests (two groups of three tests) which defined the two space factors for the college sample. In the first group of three tests, *Spatial Orientation*, *Flags*, and *Cubes*, which were incorporated to bring out a possible spatial-relations ability, the loadings were respectively .58, .44, and .43 in the factor identified as spatial-relations. In the visualization factor, the weights in these three tests were, respectively, .42, .14, and .20. Loadings in the visualization factor of .62, .52, and .52, respectively, were obtained in the three tests of the other group: *Spatial Visualization*, *Punched Holes*, and *Form Board*. For the spatial-relations factor, the weights were .44, .25, and .21, respectively. A loading of .30 or larger is probably indicative of a significant amount of variance in a given factor in view of the size of the sample (N = 360).

Reanalysis of Thurstone's Data

Fruchter's analysis. In his investigation of the nature of verbal fluency, Fruchter[1] reanalyzed a sub-matrix of the intercorrelations of twenty tests specially chosen from the battery of fifty-seven variables used by Thurstone in his pioneer study at Chicago.[12] He found two independent factors which were identified as spatial-relations and visualization. His results agree rather closely with those obtained from the college study (Rutgers) with respect to relative magnitudes of factor loadings in the definitive tests. The loadings in spatial-relations and visualization factors, respectively, for the four tests, *Cubes*, *Flags*, *Form Board*, and *Punched Holes* were .77 and .07, .72 and .29, .50 and 6.2, and .34 and .63.*

Zimmerman's analysis. Recently, Zimmerman[16] has rerotated the thirteen centroid axes for all fifty-seven variables and has defined thirteen psychologically meaningful (orthogonal) factors. Two of these factors were readily interpretable as representing independent spatial-relations and visualization abilities. It is interesting to note the comparability of the loadings in these two factors with those of Fruchter's for the same tests. Loadings in the four tests, *Cubes*, *Flags*, *Form Board*, and *Punched Holes* were for the spatial-relations and visualization factors respectively, .59 and .30, .73 and .37, .32 and .62, and .27 and .62. These sets of loadings may be compared with those obtained by Thurstone in the one spatial factor: .62, .64, .42, and .34. Other tests among Thurstone's fifty-seven which were substantially and primarily weighted in spatial-relations factor upon reanalysis were *Block Counting*, *Lozenges B*, *Surface Development*, *Pursuit*, and *Hands* with loadings, respectively, of .52, .60, .50, .51, and .55. A test which appeared to require a considerable amount of visual manipulation of figures was *Lozenges A*, with a weight of .54 in visualization.

Not too much confidence should be placed in direct comparisons between Zimmerman's and Thurstone's findings with re-

* That the magnitudes of these factor loadings are larger than those in the college study cited is due to the fact that shortened forms of Thurstone's tests were used. It is possible to estimate a change in the magnitude of factor loadings with change in length of a test if the tests are homogeneously lengthened.[5]

spect to the two space factors, since there was a noteworthy redistribution of variances for some tests on several other factors. Nevertheless, with regard to the four tests, *Cubes*, *Flags*, *Form Board*, and *Punched Holes*, which were not heavily loaded in any other intellectual factor either in Thurstone's analysis or in Zimmerman's reanalysis, it is noteworthy that the sums of squares of loadings for the factors of spatial-relations and visualization in each test, respectively, closely approximate the square of the factor loading in the spatial factor found by Thurstone. In short, the variance in Thurstone's spatial factor appears to have been resolved into two independent components.

Recommendations

There is much need for other empirical studies to test the validity of the two hypotheses. It is urged that similar batteries of tests be administered to other homogeneous samples in which such variables as age, occupational classification, amount and type of formal education, and sex membership be systematically varied. Particular attention should be directed toward ascertaining whether the spatial-relations and visualization abilities are correlated. In the three empirical studies undertaken by the writer, the amount of correlation has appeared to be negligible, although the number of tests employed is probably not sufficient to demonstrate, in a dependable manner, the presence of correlation among the abilities. It is also desirable that other hypotheses be formulated and submitted to verification through use of tests devised to reflect the operations implied by the hypotheses. These tests should be combined with reference tests of well-known factorial content, in order that other possible sources of variance in the experimental tests may be defined.

Once the spatial domain has been adequately mapped, greater emphasis can be placed upon a program of test development in which relatively pure tests can be constructed to measure the abilities identified. Even if some of the tests turn out to be factorially complex, means are available for attaining estimates of pure factor scores through use of suppression tests.[4]

Along with tests of known factorial content, numerous measures of criterion activities may be analyzed with the view of understanding better the factorial composition of these measures. Steps may then be taken to use tests which overlap to a minimum degree in the prediction of complex criteria. Such a procedure should lead, on a long range basis, to an economical program of test development.[3]

REFERENCES

1. FRUCHTER, B. 1948. The nature of verba fluency. Ed. and Psychol. Measurement **8:** 33–47.
2. GUILFORD, J. P. (Ed.) 1947. *Printed classification tests.* AAF Aviation Psychology Program Research Reports. No. 5. Washington, D. C.
3. GUILFORD, J. P. 1948. Factor analysis in a test-development program. Psychol. Rev. **5:** 79–94.
4. GUILFORD, J. P. & W. B. MICHAEL. 1948. Approaches to univocal factor scores. Psychometrika **13:** 1–22.
5. GUILFORD, J. P. & W. B. MICHAEL. Estimates of factor loadings when a test is homogeneously changed in length. (In preparation.)
6. GUILFORD, J. P. & W. S. ZIMMERMAN. 1947. The Guilford-Zimmerman aptitude survey. Beverly Hills, California: Sheridan Supply Company.
7. GUILFORD, J. P. & W. S. ZIMMERMAN. 1948. The Guilford-Zimmerman aptitude survey. *J. App. Psychol.* **32:** 24–34.
8. KELLEY, T. L. 1928. *Crossroads In the Mind of Man.* Stanford University Press.
9. MICHAEL, W. B. Contributions of factors to test variance and test validity. Psychological Monographs: General and Applied. (In press.)
10. MICHAEL, W. B., W. J. ZIMMERMAN, & J. P. GUILFORD. An investigation of two hypotheses regarding the nature of the spatial-relations and visualization factors. (To appear soon.)
11. THURSTONE, L. L. 1947. *Multiple Factor Analysis.* University of Chicago Press.
12. THURSTONE, L. L. 1938. Primary mental abilities. *Psychometric Monographs*, No. 1. University of Chicago Press.
13. THURSTONE, L. L. 1938. The perceptual factor. *Psychometrika* **3:** 1–17.
14. THURSTONE, L. L. & T. G. THURSTONE. 1941. Factorial studies of intelligence. *Psychometric Monographs*, No. 2. University of Chicago Press.

15. ZIMMERMAN, W. S. 1946. A simple graphical method for orthogonal rotation of axes. *Psychometrika*, **11**: 51–55.
16. ZIMMERMAN, W. S. 1949. Isolation, definition, and measurement of spatial-visualization abilities. Ph.D. dissertation. University of Southern California.

Note: The Section of Anthropology held a meeting on May 23, 1949, at which Doctor H. L. Shapiro, Chairman and Curator of Physical Anthropology, The American Museum of Natural History, New York, N. Y., presented a paper entitled "The Maori in Modern New Zealand," illustrated with lantern slides. This paper will be published in a later issue of the TRANSACTIONS.

*DIVISION OF MYCOLOGY**

INFLUENCE OF NUTRITION ON GROWTH AND MORPHOLOGY OF THE DERMATOPHYTES†

By LUCILLE K. GEORG§

Nutritional studies of the dermatophytes have been undertaken for the purpose of determining the optimal requirements for growth and spore production in these fungi. Such a study is important in order to improve both isolation and identification techniques and, thus, increase the certainty of diagnosis in ringworm disease.

Many classifications have been proposed for the dermatophytes, the first being that of Sabouraud, which was based primarily on the clinical picture or type of lesion. Others took into consideration more botanical characteristics. In 1934, Emmons,[1] in an attempt to simplify the study of these forms, proposed a classification which is based largely on the type of macroconidium, or large septate spore, characteristic of these fungi. The production of spores has, thus, become very important in the identification of the dermatophytes.

Difco, Sabouraud's dextrose or maltose agars, now used extensively for isolation purposes as well as for the production of characteristic colonies, have not been found completely satisfactory for the study of microscopic characteristics, and a variety of media such as corn meal or potato dextrose agar have come into use. However, for those forms which produce macroconidia sparsely, such as *Microsporum audouini* and many of the trichophyton species, these media are inadequate, and new media are constantly sought in order to induce or increase spore production in these strains.

A great stimulus was given to the study by the introduction of the "natural media of polysaccharide base" by Langeron and Milochevitch[2] in 1930. On these media which consisted of whole grains of wheat, barley, corn, and oats, they were able to induce the formation of spirals, microconidia, and macroconidia in many strains of trichophytons which had not previously shown these structures on the usual Sabouraud's media.

The stimulation of growth and spore production on natural media suggested that these substances are rich in growth factors, vitamins, and amino acids which may be required by these organisms for their normal development. Recent studies, using synthetic, chemically-defined media, by Robbins *et al.*,[3] Schopfer and Blumer,[4] Burkeholder and Moyer,[5] Mackinnon and Artagaveytia-Allende,[6] Georg,[7] and others have shown, in fact, that many of these fungi actually have deficiencies for certain of the vitamins and amino acids and are stimulated by other growth factors present in natural products such as peptones, liver, and yeast extracts.

Robbins and Ma,[8] in their study of "Growth Factors for *Trichophyton menta-*

* Doctor Samuel M. Peck has requested the Editor of TRANSACTIONS to publish a corrected title for his paper, read before the Division of Mycology of the Academy on January 28, 1949, and presented by him for publication under the title: "A New Method for the Evaluation of Antifungicidal Chemicals." The author desires to correct this title to read: "A New Method for the Evaluation of Antifungal Chemicals."

† This study was accomplished through a grant-in-aid from the U. S. Public Health Service.

§ Department of Dermatology, College of Physicians and Surgeons, Columbia University, New York, N. Y. This paper, illustrated with lantern slides, was presented at the meeting of the Division on May 27, 1949.

grophytes," have shown that the normal sporulating form of this organism requires at least one amino acid for growth. Inorganic nitrogen in the form of NH_4NO_3 is almost completely unavailable to this fungus. However, the culture grows more rapidly with peptone, casein hydrolysate, and other natural products than with asparagine as the only source of nitrogen. No evidence was obtained for the requirement of any particular amino acid, any one of 14 tested being able to act as a source of nitrogen, and the fungus was autotrophic as far as the vitamins were concerned. These authors further demonstrated that the mutant or pleomorphic forms of *T. mentagrophytes* (those forms which produce rapidly growing, white, fluffy colonies composed largely of sterile mycelia) were physiologically different from the normal sporulating form. Not only can the pleomorphic form utilize asparagine much more readily, but it has the ability to use inorganic nitrogen such as ammonia.

Conant,[9] in his study of the microsporums, discovered that species of animal origin (*M. canis and M. gypseum*) grew profusely on polished rice grains and produced macroconidia in abundance, but that *Microsporum audouini*, on the other hand, grew poorly, showing no aerial growth and producing no macroconidia. This suggested that *M. audouini* was a deficient organism. Other findings led to the same conclusion. Benedek[10] found accidentally that a culture of *M. audouini*, contaminated with a bacillus which he named *Bacillus weidmaniensis*, was able to grow well on polished rice grains and produced macroconidia. Hazen[11] demonstrated that yeast extract added to honey agar resulted in a marked increase in the vegetative growth of *M. audouini* and in the production of macroconidia. The author suggested that growth factors essential to the development of *M. audouini*, present in yeast extract and produced by *B. weidmaniensis*, are probably identical. Hazen recommends a honey-peptone agar with 5 mg. yeast extract per ml. of medium for the production of macroconidia in *M. audouini*.

Benham[12] has studied the effect of nutrition on the development of macroconidia in *Trichophyton rubrum*. Here, as with the other dermatophytes, the production of the characteristic macroconidia is of great importance in the identification of the fungus. The macroconidium of *T. rubrum* are characterized as long, narrow spores of 3 to 10 septa, with nearly straight, parallel sides and blunt or pointed ends. These from the most distinctive feature of this species. They are, however, inconsistently produced on the usual media. By the use of a heart-infusion-tryptose agar, Blood Agar Base (Difco), Benham was able to induce the production of macroconidia in 49 of 50 strains studied on this medium. Blood Agar Base differs from Sabouraud's medium in reaction, having a pH of 6.8, in the absence of sugar, and in containing beef heart infusion and "tryptose," a meat digest with a higher percentage of proteose than the usual peptones. Tryptose appears to be the ingredient responsible for the production of macroconidia of *T. rubrum* on this medium.

The group of so-called "faviform trichophytons" has been of particular interest in the study of the relationship of nutrition to growth and production of spores. The organisms of this group, in which we include *Trichophyton schoenleini*, *Trichophyton faviforme*, and *Trichophyton violaceum*, have been characterized in the past largely by their deficiencies. On the usual Sabouraud's dextrose agar, they produce slow-growing, usually glabrous colonies consisting of irregular mycelia and many chlamydospores. However, by the use of whole grain media, Langeron and Milochevitch[2] have demonstrated that a rapid downy to fluffy growth with microconidia may be obtained with some of these strains.

Trichophyton schoenleini, the causative agent of the disease favus, is the most rapidly growing member of this group. On Sabouraud's dextrose agar, the average strain will produce a colony of 30 to 50

millimeters in diameter by six weeks. The growth is frequently heaped in the center and may show heavy folding. Some strains stay moist and glabrous, but most develop a small amount of white powder or short, white down over the surface of the colony. The mycelium is extremely irregular, showing the characteristic "chandeliers" and many chlamydospores.

In a study of six strains of *Trichophyton schoenleini*, using chemically-defined, vitamin-free media, we found that all of the strains grew well on basal dextrose agars with either NH_4NO_3 or acid digest, vitamin-free casein as source of nitrogen. The growth was not stimulated by any of a large series of vitamins tested. Some increase in growth and production of an aerial mycelium was, however, observed by the addition of heart-infusion or rich peptones such as Bacto-Tryptose. Culture on whole grains also improves the growth and a few microconidia were obtained on such media. These natural substances could not be substituted for by large amounts of any of the vitamins tested or by casein hydrolysate. Apparently, this is not a vitamin- or amino acid-deficient organism, although it does require some factors present in natural products for the production of spores.

Trichophyton faviforme, under which we include the album, discoides, and ochraceum species of Sabouraud, has, on the other hand, been shown to have complete deficiences for certain vitamins. This organism, which produces a large-spored, ectothrix invasion of the hair, is a common agent of ringworm in cattle and horses which is frequently transmitted to man. These organisms grow extremely slowly and poorly on the usual Sabouraud's dextrose agar, often producing colonies of not more than 10 millimeters in diameter after six weeks. Microscopically like *Trichophyton schoenleini*, the only structures seen are highly irregular mycelia and large numbers of chlamydospores.

Studies on synthetic, vitamin-free media by Robbins, Mackinnon, and Ma[3] with one of these large-spored, ectothrix organisms of animal origin, *Trichophyton discoides*, indicated that this fungus required three vitamins for growth: i-inositol, thiamine, and pyridoxine. Mackinnon and Artagaveytia-Allende[6] studied a series of 12 strains and found them to require only two vitamins, inositol and thiamine.

In a series of 28 cases of suppurative ringworm contracted from cattle, caused by *Trichophyton faviforme* and described by Fowle and Georg, [13] it was noted that the usual Sabouraud's dextrose agar was inadequate for isolation of the causative fungi in many of these cases. These authors recommended a heart-infusion-tryptose agar such as Blood Agar Base (Difco), to which has been added 0.1 mg. thiamine per 100 ml. Antibiotics such as pencillin and streptomycin may be added to inhibit bacterial growth. On such media, *Trichophyton faviforme* may be readily isolated from most cases. The colonies develop rapidly, with a downy to fluffy surface growth with large numbers of microconidia and occasionally a few macroconidia. Studies[7] with single spore isolates indicated that the three colony types described by Sabouraud as *T. album*, *discoides*, and *ochraceum* could be obtained from a single spore. It was recommended that *T. faviforme* be given as the type species of this group.

Nutritional studies by Georg[7] on synthetic, vitamin-free media with a group of sixteen recently isolated strains, as well as four stock cultures from the Culture Bureau in Holland, gave the following results: Eighteen of twenty strains of *Trichophyton faviforme* showed complete vitamin deficiences for inositol and molecular thiamine. Inositol produced some slight effect alone, but allowed only a very limited amount of growth. The minimal effective concentration was 5 gamma per 5 ml. of NH_4NO_3 broth. Thiamine showed no action alone, but, in the presence of an adequate supply of inositol, the amount of growth was proportional to the amount of thiamine present. The minimal effective concentration of thiamine was 0.01 gamma per 5 ml. of NH_4NO_3

broth. On synthetic, vitamin-free agar, with either NH_4NO_3 or acid-digest casein as nitrogen source and adequate supplies of the required vitamins, *T. faviforme* will produce rapidly growing colonies with downy to fluffy surface growth with large numbers of microconidia and, in most instances, macroconidia.

Recently, we have made a study of a third member of this group, *Trichophyton violaceum*, a common cause of chronic dermatophytosis and scalp ringworm in northern Africa, Spain, Russia, and China. The disease occasionally appears in the United States, and the characteristic endothrix invasion of the hair is well known. This fungus grows very slowly on Sabouraud's dextrose agar, producing small, moist, glabrous, usually highly verrucose colonies with a violet to deep purple pigment. Microscopically it is composed of a poorly-formed, irregular, twisted mycelium and many chlamydospores. The cultures tend to die unless frequently transferred to fresh media, and, on subculture, undergo changes of two types: (1) pleomorphism, or development of rapidly growing areas of white, woolly, sterile mycelia which may eventually cover the entire colony; and (2) development of lightly pigmented or white sectors of flat, spreading growth covered with a short, fine down which may occasionally bear a few very delicate microconidia.

There is some evidence in the literature to indicate that *Trichophyton violaceum* is a "deficient fungus" and requires special media for good growth and the production of spores. Burkeholder and Moyer[5] have shown that a strain of *T. violaceum* which they studied was deficient for thiamine and that its growth was stimulated by additions of liver and peptones.

We have recently studied a series of eleven strains of *Trichophyton violaceum*. These cultures showed all variations in colony form, and ten of the eleven cultures showed characteristic microscopic morphology on Sabouraud's dextrose agar. The more recently isolated, moist, glabrous colonies were composed largely of chlamydospores and fragments of irregular hyphae. The older stock cultures, most of which had lost the purple pigment and showed some downiness, had a very fine mycelium which showed clubbed ends and balloon-like chlamydospores. Some of the colonies showed spiny surface growth which consisted of ropes of twisted mycelia resembling coremia. By a diligent search in some of these cultures, mycelia could be found which bore a few delicate microconidia.

In contrast to these ten typical-appearing strains, a white fluffy strain, obtained from the Culture Bureau for Fungi, Delft, Holland, gave the appearance of a completely pleomorphic culture. However, on examination it was found that the aerial mycelium was covered with many microconidia. This culture, #565, proved to be completely different in its nutritional requirements from the other ten strains studied, and perhaps this accounted for the difference in its morphological characteristics.

The ten typical strains could be maintained on a vitamin-free dextrose agar with either inorganic or organic nitrogen. Availability of nitrogen was in the following order: acid-digest casein $>$ NH_4NO_3 $>$ NH_4Cl $>$ $NaNO_3$. Development on such media, however, was very restricted and consisted of a thin submerged growth. Increasing the amount of available nitrogen did not increase the amount of this growth.

A survey of the vitamin requirements of these strains indicated that the growth of the ten similar strains was greatly increased by the addition of thiamine to either the NH_4NO_3 or the acid-digest casein agar. The only other vitamin which had a stimulatory effect was para-amino-benzoic acid. The effect was slight and not apparent unless 300 to 500 gamma were added to 5 ml. NH_4NO_3 broth. (That this effect was not due to contamination with thiamine was determined by testing the P.A.B. solution with washed spores of *Phycomyces blakesleeanus*.) The stimulatory effect of

thiamine ranged from 0.002 gamma to 0.5 gamma in 5 ml. NH_4NO_3 broth. Further additions caused no increased growth, but even very large doses had no inhibitory effect. Only the pyrimidine portion of the molecule was active. Molecular thiamine was replaced by an equi-molar amount of 2 - methyl - 5 - ethoxymethyl - 6 - amino - pyrimidine. Oxalacetate was found to substitute partially in a manner similar to that observed by Benham[14] for *Pityrosporum ovale*, an organism which is also stimulated by thiamine.

The addition of maximal effective dosages of thiamine to NH_4NO_3 or acid-digest casein agars allowed rapid growth of these strains with downy to fluffy aerial mycelia with numerous microconidia. Although the strains grew well on thiamine-enriched NH_4NO_3 agar, a more rapid and heavier growth was obtained on thiamine-enriched casein agar. On a 0.5 per cent casein medium with 50 gamma thiamine per ml., heavy folded colonies were obtained with considerable surface down. Not only were microconidia produced in large numbers, occurring along the mycelium (*en thyrse*), and terminally in large clusters (*en grappe*), but the macroconidia of this species were observed for the first time. The macroconidia observed were 2, to, 5 celled, smooth-walled, hyaline, elongate structures borne both terminally and laterally on the mycelium. The walls are thin and frequently indented at the septae. The large majority are slightly clavate and resemble rather closely the macroconidia of *T. mentagrophytes*.

Strain ✻365 showed no growth on NH_4NO_3 basal agar even when large amounts of thiamine or any combination of the vitamins were added. It grew well, however, on vitamin-free casein agar. It was apparent that the deficiency here was for some amino acid or combination of amino acids. On a 0.5 per cent casein agar, a rapidly growing, heavily folded colony was obtained with a fluffy aerial growth containing large numbers of microconidia and also macroconidia similar to those produced by the thiamine-deficient strains. By testing amino acids in groups and singly, it was found that growth on the NH_4NO_3 agar could take place only when the amino acid histidine was added. The effective concentration ranged from 0.0003 mg. (300 gamma) to 0.08 mg. in 5 ml. NH_4NO_3 broth. With doses over 0.15 mg., toxic effects were observed. Histidine, alone, only allowed a meagre growth on NH_4NO_3 agar, which indicated that the addition of other amino acids would be necessary to produce growth comparable to that observed on the casein agar. The remaining amino acids were added singly and in combinations with histidine. The most available fall in the following order: glutamic > aspartic > arginine > lysine. Hydroxyproline was found to have a partial inhibitory effect in a dilution as high as M/1000. Complete inhibition occurred with 3 mg. hydroxyproline in 5 ml. NH_4NO_3 broth containing 10 gamma of histidine. This inhibition could be overcome by adding proline to the medium. Robbins and McVeigh[15] have reported a similar inhibition of the growth of *Trichophyton mentagrophytes* by hydroxyproline which could be overcome by proline. It seems apparent that these hydroxyproline-sensitive strains require proline in their metabolism.

In the presence of thiamine, the ten thiamine-deficient strains showed stimulation by amino acids. Aspartic acid was the most readily available. Other amino acids with stimulatory effects in a dilution of M/1000 were arginine, glutamic, histidine, phenylalanine, and proline. No inhibitory effect due to hydroxyproline was observed when these strains were grown in NH_4NO_3 broth. In casein broth, however, slight inhibitory effect was observed with 15 mg. hydroxyproline. This corresponds to the findings of Robbins and McVeigh,[15] who showed that the normal form of *Trichophyton mentagrophytes* (the amino acid-requiring form) was much more sensitive to hydroxyproline than the pleomorphic form which can utilize inorganic nitrogen.

The fact that one of the eleven strains

studied was physiologically different from the rest suggested that this might be an unusual mutant form or even a different species. However, by inoculating a spore suspension of this culture into the shaven side of a rabbit, an infection with erythema, scaling, and endothrix invasion of the hairs was observed. The culture recovered was identical to the culture inoculated, except that a purple pigment was visible on the reverse side of the colony. The type of infection and the recovery of the fungus with purple pigment indicated to us that this was indeed *Trichophyton violaceum*. This was further substantiated by that fact that at one time, daughter colonies found scattered around a colony of the white, fluffy culture appeared to be of the more usual purple, glabrous type. These were subcultured and a pure glabrous dark purple form was obtained. This culture, # 365a, was apparently a back mutation to the common form. Upon study, it was found not only to have lost its deficiency for histidine, but to have acquired a deficiency for thiamine. This strain proved to be identical to the ten other thiamine-deficient strains studied.

Summary

(1) The production of characteristic spores—particularly the macroconidium—is necessary in the identification of the dermatophytes.

(2) It has been shown that the presence of certain vitamins, amino acids, and other growth factors present in natural products are necessary for good growth and the production of spores in many of the dermatophyte species. This is true for *Microsporum audouini*, *Trichophyton rubrum*, *Trichophyton schoenleini*, *Trichophyton faviforme*, and *Trichophyton violaceum*.

(3) The macroconidia of *Trichophyton violaceum* have been described for the first time.

(4) It is suggested that for certain forms, which show complete or almost complete deficiencies, special enriched media be used for isolation and maintenance of these cultures.

(5) Further studies of this type may be of value in the promotion of good growth and the production of spores in other dermatophyte species.

BIBLIOGRAPHY

1. Emmons, C. W. 1932. Pleomorphism and variation in the dermatophytes. Arch. Dermat. & Syph. **25:** 987–1001.
2. Langeron, M. & S. Milochevitch. 1930. Morphologie de dermatophytes sur milieux naturels à base de polysaccharides des essai de classification. Ann. de parasitol. **8:** 465–508.
3. Robbins, W. J., J. E. Mackinnon, & R. Ma. 1942. Vitamin deficiencies of *Trichophyton discoides*. Bull. Torrey Club **69:** 509–521.
4. Schopfer, W. H. & S. Blumer. 1942. Recherches sur le besoin en facteurs de croissance vitaminiques et le pourvoir de synthese d'un trichophyton. Soc. phys. hist. nat. genève **59:** 106–112.
5. Burkeholder, P. R. & D. Moyer. 1943. Vitamin deficiencies of fifty yeasts and molds. Bull. Torrey Club **70:** 372–377.
6. Mackinnon, J. E. & R. C. Artagaveytia-Allende. 1948. Vitamin deficiencies of seven strains of ectothrix, large-spored trichophytons isolated from man and cattle. J. Bact. **56:** 91–96.
7. Georg. L. K. Nutritional requirements of the faviform trichophytons. Ann. N. Y. Acad. Sci. In press.
8. Robbins, W. J. & R. Ma. 1945. Growth factors for *Trichophyton mentagrophytes*, Am. J. Bot. **32:** 509–523.
9. Conant, N. F. 1936. Studies in the genus *Microsporum*. Cultural Studies. Arch. Dermat. & Syph. **33:** 665–683.
10. Benedek, T. 1943. Unilateral stimulation of *Microsporum audouini* by a new species of bacillus. Mycologia. **35:** 222–242.
11. Hazen, E. L. 1947. *Microsporum audouini:* The effect of yeast extract, thiamine, pyridoxine, and *Bacillus weidmaniensis* on the colony characteristics and macroconidial formation. Mycologia. **39:** 200–209.
12. Benham, R. W. 1948. Effect of nutrition on growth and morphology of the dermatophytes. 1. Development of macroconidia in *Trichophyton rubrum*. Mycologia. **40:** 232–240.
13. Fowle, L. P. & L. K. Georg. 1947. Suppurative ringworm contracted from cattle. Arch. Dermat. & Syph. **56:** 780–793.
14. Benham, R. W. 1945. Pityrosporum ovale—A lipophilic fungus. Thiamine and oxalacetic acid as growth factors. Proc. Soc. Exper. Biol. & Med. **58:** 199–201.
15. Robbins, W. J. & I. McVeigh. 1946. Effect of hydroxyproline on *Trichophyton mentagrophytes* and other fungi. Am. J. Bot. **33:** 638–647.